PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/00, 38/32, 38/46 — A1

(11) International Publication Number: WO 96/11016

(43) International Publication Date: 18 April 1996 (18.04.96)

(21) International Application Number: PCT/US95/12543

(22) International Filing Date: 6 October 1995 (06.10.95)

(30) Priority Data:

| | | |
|---|---|---|
| 08/318,994 | 6 October 1994 (06.10.94) | US |
| 08/483,572 | 7 June 1995 (07.06.95) | US |


(71) Applicants: ALPHA 1 BIOMEDICALS, INC. [US/US]; Two Democracy Center, Suite 1200, 6903 Rockledge Drive, Bethesda, MD 20817-1818 (US). THE GEORGE WASHINGTON UNIVERSITY MEDICAL CENTER [US/US]; 2300 Eye Street, N.W., Washington, DC 20037 (US).

(72) Inventors: CROCKFORD, David, R.; 113 Record Street, Frederick, MD 21701 (US). RUBIN, Bruce, K.; 1850 North Signal Hills Drive, Kirkwood, MO 63122 (US). BERMAN, Michael, L.; 8824 Watts Mine Terrace, Potomac, MD 20854 (US). GOLDSTEIN, Allan, L.; 6407 Bradley Boulevard, Bethesda, MD 20817 (US). BAUMAN, Christian; 14355 Georgia Avenue, Silver Spring, MD 20906 (US). KATER, Arnon; 950-25th Street, N.W., 514 South, Washington, DC 20037 (US).

(74) Agents: REPPER, George, R. et al.; Rothwell, Figg, Ernst & Kurz, 555 13th Street, N.W. #701 East, Washington, DC 20004 (US).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published

*With international search report.*

*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: TREATMENT OF OBSTRUCTIVE AIRWAY DISEASE BY ADMINISTERING THYMOSIN $\beta_4$, OR COADMINISTRATION OF THYMOSIN $\beta_4$ AND DNase I

(57) Abstract

A method of treating obstructive airway disease (OAD) such as cystic fibrosis involves contacting OAD sputum with a viscoelasticity-reducing amount of Thymosin $\beta_4$, or a combination of Thymosin $\beta_4$ and DNase I.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|---|---|---|---|---|---|
| AT | Austria | GB | United Kingdom | MR | Mauritania |
| AU | Australia | GE | Georgia | MW | Malawi |
| BB | Barbados | GN | Guinea | NE | Niger |
| BE | Belgium | GR | Greece | NL | Netherlands |
| BF | Burkina Faso | HU | Hungary | NO | Norway |
| BG | Bulgaria | IE | Ireland | NZ | New Zealand |
| BJ | Benin | IT | Italy | PL | Poland |
| BR | Brazil | JP | Japan | PT | Portugal |
| BY | Belarus | KE | Kenya | RO | Romania |
| CA | Canada | KG | Kyrgystan | RU | Russian Federation |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan |
| CG | Congo | | | SE | Sweden |
| CH | Switzerland | KR | Republic of Korea | SI | Slovenia |
| CI | Côte d'Ivoire | KZ | Kazakhstan | SK | Slovakia |
| CM | Cameroon | LI | Liechtenstein | SN | Senegal |
| CN | China | LK | Sri Lanka | TD | Chad |
| CS | Czechoslovakia | LU | Luxembourg | TG | Togo |
| CZ | Czech Republic | LV | Latvia | TJ | Tajikistan |
| DE | Germany | MC | Monaco | TT | Trinidad and Tobago |
| DK | Denmark | MD | Republic of Moldova | UA | Ukraine |
| ES | Spain | MG | Madagascar | US | United States of America |
| FI | Finland | ML | Mali | UZ | Uzbekistan |
| FR | France | MN | Mongolia | VN | Viet Nam |
| GA | Gabon | | | | |

# TREATMENT OF OBSTRUCTIVE AIRWAY DISEASE BY ADMINISTERING THYMOSIN B4, OR COADMINISTRATION OF THYMOSIN B4 AND DNase I

The present invention relates to methods and compositions for treating obstructive airway disease in mammals.

## Description of Background Art

Obstructive airway disease (OAD) encompasses a number of respiratory disorders and is associated with viscoelastic secretions or exudate (sputum) in the patient's airways which contribute significantly to respiratory distress and may also contribute to progressive lung destruction.

OAD sputum is a complex material known to contain DNA and other materials, including proteins such as actin. OAD sputum is produced in patients with cystic fibrosis (CF), and may also be produced in patients with various forms of bronchitis, bronchiolitis, pneumonia, asthma, sinusitis, bronchorrhea, adult respiratory distress syndrome (ARDS), empyema, bronchiectasis, bronchocoele and emphysema.

Recombinant human DNase I (rhDNase I) has been reported to diminish viscosity of CF sputum *in vitro* (Shak et al., *PNAS USA*, *87*:9188-9192 [1990]). Human DNase I has been approved in the United States for treating certain CF patients.

Thymosin $\beta_4$ ($T\beta_4$) is a peptide which has been reported as containing 43 amino acids. Amino acid sequence information on $T\beta_4$ is disclosed in U.S. Patent No. 4,297,276, herein incorporated by reference.

$T\beta_4$ has been found to be present in numerous tissue types in mammals and has also been implicated in a wide variety of cellular and physiological processes including actin sequestration within cells, inducing terminal deoxynucleotidyl transferase activity of bone marrow cells, stimulating secretion of hypothalamic luteinizing hormone releasing hormone and luteinizing

hormone, inhibiting migration and enhancing antigen presentation of macrophages, and inducing phenotypic changes in T-cell lines *in vitro*.

There remains a need in the art for new methods of treating OAD.

## Summary of the Invention

In accordance with the present invention, methods and compositions for treating OAD in a mammal utilize $T\beta_4$ or co-administerion of $T\beta_4$ and DNase I to the mammal.

## Brief Description of the Drawings

Fig. 1 shows amino acid sequences ID Nos. 1-16, respectively, of $T\beta_4$ compounds useful in the invention.

Fig. 2A is a bar graph showing effect of Tβ4 on OAD sputum viscosity.

Fig. 2B is a bar graph showing the effect of $T\beta_4$ and DNase I on storage modulus of OAD sputum at 1 radian/sec.

Fig. 3A is a graph showing $T\beta_4$ and actin depolymerization.

Fig. 3B is a bar graph showing the effect of $T\beta_4$ and DNase I on storage modulus of OAD sputum at 100 radian/sec.

Fig. 4 is a bar graph showing the effect of $T\beta_4$ and DNase I on the vectorial sum of storage modulus and loss modulus of OAD sputum at 100 radian/sec.

Fig. 5 is a bar graph showing the effect of $T\beta_4$ and DNase I on OAD sputum loss modulus at 1 radian/sec.

Fig. 6 is a bar graph showing the effect of $T\beta_4$ and DNase I on the ratio of loss modulus to storage modulus of OAD sputum at 100 radian/sec.

Fig. 7 is a bar graph showing the effect of $T\beta_4$ and DNase I of the ratio of loss modulus to storage modulus of OAD sputum at 1 radian/sec.

Fig. 8 is a bar graph showing the effect of $T\beta_4$ and DNase I on the vectorial sum of storage modulus and loss modulus of surface OAD sputum at 1 radian/sec.

Fig. 9 is a bar graph showing the effect of $T\beta_4$ and DNase I on the vectorial sum of storage modulus and loss modulus of internal OAD sputum at 1 radian/sec.

Fig. 10 is a bar graph showing the effect of $T\beta_4$ and DNase I on OAD sputum loss modulus at 100 radian/sec.

## Description of the Preferred Embodiments

One embodiment of the present invention involves administration of $T\beta_4$ to mammals to treat OAD including respiratory disorders such as acute and chronic respiratory distress syndromes, chronic bronchitis, asthma, emphysema and cystic fibrosis. Without being bound to any particular theory, it is believed that these respiratory disorders may be associated with excess actin polymerization, i.e., polymerization of G-actin (monomeric form) into F-actin.

The terms "Thymosin $\beta_4$" and "$T\beta_4$" refer to peptides having the amino acid sequence disclosed in U.S. Patent No. 4,297,276, supra.

According to one aspect of the present invention, effective amounts of $T\beta_4$ are administered to a mammal, such as a human patient having a respiratory disorder, so as to depolymerize F-actin, or alternatively prevent G-actin polymerization. Such effective amounts can be referred to as actin-antipolymerizing amounts.

Thus, $T\beta_4$ can be utilized in accordance with the present invention to treat respiratory disorders mediated by excess actin polymerization. Accordingly, $T\beta_4$ can be utilized to treat patients having a respiratory disorder selected from the group consisting of acute and chronic respiratory distress syndromes, and advantageously can be utilized to treat chronic bronchitis, asthma, emphysema and cystic fibrosis.

A preferred embodiment of the present invention involves treating cystic fibrosis with $T\beta_4$. Patients with cystic fibrosis accumulate thick secretions (sputum) in their airways that cause progressive pulmonary destruction. Cystic fibrosis sputum is a complex material, but a major cause of its thick consistency is pus, derived from masses of degenerating leukocytes.

Treatment of cystic fibrosis in accordance with this embodiment involves administering to a CF patient a sputum viscosity-reducing amount of $T\beta_4$. Since filamentous actin may be responsible for at least some of the viscosity of cystic fibrosis sputum, the amount of $T\beta_4$ administered to a CF patient may be characterized as an actin-antipolymerizing amount thereof.

Effective dosage amounts of $T\beta_4$ for treatment of respiratory disorders including acute respiratory distress syndrome, chronic bronchitis, asthma, emphysema and cystic fibrosis, are generally less than about 10 mg/kg of body weight of the recipient, and are preferably within the range of from about 100 μg/kg to about 1 mg/kg. A dose can be administered to the patient daily, one or more times per day of administration, e.g., one to six times or more per day, and doses can be administered one or more days per week, e.g., two, three, four, five, six or seven days per week. The precise dose administered will depend on the age, condition and other factors of the recipient.

According to preferred embodiments of the present invention, compositions containing $T\beta_4$ may be formulated in a conventional manner for administration by any suitable route. Preferred methods of administration include inhalation of a composition containing $T\beta_4$ into the patients' lungs through the mouth and/or nose. In this embodiment, the $T\beta_4$ composition can be an aerosol.

Other preferred routes of administration may include injection/infusion (including parenteral, subcutaneous, intramuscular, intravenous and intradermal). It will be appreciated that the preferred route may vary with the condition and age of the recipient.

Alternatively, oral or other routes of administration may be utilized.

In preferred embodiments, $T\beta_4$ is administered as part of a pharmaceutical formulation. The formulations of the present invention comprise $T\beta_4$ together with one or more pharmaceutically acceptable carriers. The carrier(s) are "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for inhalation, injection/infusion (including parenteral, subcutaneous, intramuscular, intravenous and intradermal) or other routes of administration. The formulations may conveniently be presented in unit dosage form, including aerosol, liquid, solid, or powered unit dosage form, and may be prepared by any suitable pharmaceutical method.

Such methods include, but are not limited to, the step of bringing into association $T\beta_4$ with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association $T\beta_4$ with liquid carriers or finely divided solid carriers or both.

Formulations of the present invention suitable for oral administration may be presented as discrete units each containing a predetermined amount of $T\beta_4$; as an aerosol; as a powder; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion, etc.

Aerosols suitable for inhalation generally contain liquid or solid particles of less than about 100 microns in size, preferably less than about 50 microns in size, and more preferably less than about 25 microns in size. In particularly preferred embodiments, the aerosol particle size is in the range of about 0.1-10 microns, more preferable less than about 4 microns, and most preferable about 0.1-3 microns.

Formulations suitable for injection/infusion, or parenteral administration, include aqueous and non-aqueous sterile injection solutions which may optionally contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with body fluids of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use.

Extemporaneous aerosol or injection solutions or suspensions may be prepared from solid or liquid formulations of the kind previously described.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other suitable agents having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

According to one embodiment of the present invention, the viscoelasticity of mammalian OAD sputum is reduced by contacting OAD sputum with a viscoelasticity-reducing amount of a combination of $T\beta_4$ and DNase I.

The term "DNase I" refers to peptides having the amino acid sequence disclosed in Shak et al., *supra*, and elsewhere.

Without being bound to any particular theory, the high viscoelasticity of OAD sputum may be due to interaction between DNA and excess polymerized actin (F-actin) formed by polymerization of G-actin (monomeric actin).

While DNase I is known to reduce viscosity of sputum from CF patients, and $T\beta_4$ is a known actin sequestration protein, it has surprisingly been found that combinations of $T\beta_4$ and DNase I reduce OAD sputum viscoelasticity to a significantly greater extent than would be expected from corresponding individual amounts of $T\beta_4$ and DNase I.

A preferred embodiment of the present invention involves treating OAD by contacting OAD sputum with a viscoelasticity-reducing amount of a combination of $T\beta_4$ and DNase I. Patients with obstructive airway disease such as CF accumulate thick secretions of sputum in their airways that cause progressive pulmonary destruction. As noted above, OAD sputum is a complex material, but a major cause of its thick consistency in CF is pus, derived from masses of degenerating leukocytes. Treatment of OAD in accordance with this embodiment involves administering to a human OAD patient a pharmaceutical formulation including a viscoelasticity-reducing amount of a combination of $T\beta_4$ and DNase I in a pharmaceutically-acceptable liquid carrier. In preferred embodiments, the liquid carrier is an aqueous carrier, *e.g.*, water for injection,

and may contain anti-oxidants, buffers, bacteriostats, antibiotics, solutes, and/or other ingredients.

In particularly preferred embodiments, the inventive pharmaceutical formulation including $T\beta_4$ and DNase I is administered to an OAD patient by introducing the formulation into one or more airways of the patient so as to contact the formulation with OAD sputum present in the patient's airways. Preferred methods of administration including inhalation of the inventive pharmaceutical formulation into the patient's lungs through the patient's mouth and/or nose. In this embodiment, the inventive formulation can be an aerosol. Injectable or infusible compositions may also be administered, either concurrently, separately or alone.

Effective amounts of $T\beta_4$ are amounts sufficient to depolymerize F-actin in OAD sputum or, alternatively, prevent G-actin polymerization in OAD sputum. Such effective amounts can be referred to as actin-antipolymerizing amounts.

Effective amounts of DNase I are capable of further reducing the viscoelasticity of OAD sputum in conjunction with $T\beta_4$ by cleaving elongate strands of DNA present in OAD sputum and/or further preventing polymerization of actin. $T\beta_4$ may also enhance DNase I activity in cleaving DNA by preventing DNase I from binding to actin.

In preferred embodiments, the $T\beta_4$ and DNase I are each present in the inventive pharmaceutical formulation in a respective ratio of from about 1:2 to about 2:1, more preferably at a ratio of about 1:1.

In accordance with one embodiment, the concentration of $T\beta_4$ in the inventive formulation is within a range of about 0.1-200 mcg/ml, preferably about 0.3-150 mcg/ml, more preferably about 0.5-30 mcg/ml, even more preferably about 1-10 mcg/ml, still more preferably about 2-5 mcg/ml, and most preferably about 3 mcg/ml.

The concentration of DNase I in the inventive formulation can be within a range of about 0.1-200 mcg/ml, preferably about 0.3-150 mcg/ml, more

preferably about 0.5-30 mcg/ml, even more preferably about 1-10 mcg/ml, still more preferably about 2-5 mcg/ml, and most preferably about 3 mcg/ml.

In accordance with another embodiment, the concentration of $T\beta_4$ in the inventive formulation is within a range of about 0.1-10 mg/ml, preferably about 0.3-7 mg/ml, more preferably about 0.5-5 mg/ml.

The concentration of DNase I in the inventive formulation can be within a range of about 0.1-10 mg/ml, preferably about 0.3-7 mg/ml, more preferably about 0.5-5 mg/ml.

In accordance with one aspect, the inventive formulation is administered to an OAD patient so that about 0.5-10 mg per day of each of $T\beta_4$ and DNase I is administered to the patient, and preferably about 2.5-5 mg per day of each is administered to the patient. The daily dose can be administered to the patient all at once, or portions of the daily dose can be administered in a regimen spread over the day, *e.g.*, in increments of one to six times or more per day. Furthermore, doses can be administered one or more days per week, *e.g.*, 2, 3, 4, 5, 6 or 7 days per week. The precise dose administered will depend on the age, condition and other factors of the recipient.

The present invention is also directed to pharmaceutical formulations comprising an OAD sputum viscoelasticity-reducing amount of a combination of $T\beta_4$ and DNase I. As indicated above, the preferred inventive formulations are aerosols suitable for inhalation. Such aerosols generally contain particles (preferably liquid) of less than about 100 $\mu$ in size, preferably less than about 50 $\mu$ in size, and more preferably less than about 25 $\mu$ in size. In particularly preferred embodiments, the aerosol particle size is in the range of about 0.1-10 $\mu$, more preferably less than about 4 $\mu$, and most preferably about 0.1-3 $\mu$.

The invention is applicable to native (i.e., naturally occurring) $T\beta_4$ as well as synthetic $T\beta_4$ and recombinant $T\beta_4$ having the amino acid sequence of native $T\beta_4$, biologically active amino acid sequences substantially similar thereto, or a biologically active abbreviated sequence form thereof, and their biologically active analogs (including muteins) having substituted, deleted, elongated, replaced, or otherwise modified sequences which possess bioactivity

substantially similar to that of native $T\beta_4$. Representative sequences are shown in Fig. 1.

The invention also is applicable to native (*i.e.*, naturally-occurring) human DNase I, as well as other DNase I peptides which are compatible with human patients, along with synthetic DNase I and recombinant DNase I having an amino acid sequence of native DNase I, biologically-active amino acid sequences substantially similar thereto, or a biologically-active abbreviated sequence form thereof, and their biologically-active analogs (including muteins) having substituted, deleted, elongated, replaced or other modified sequences which posses bioactivity substantially similar to that of human DNase I.

The following examples are for illustrative purposes only, and are not to be construed in a limiting sense.

## Example 1

Synthetic $T\beta_4$ was provided by Alpha 1 Biomedicals, Inc. (Two Democracy Center, 6903 Rockledge Drive, Ste. 1200, Bethesda, Maryland 20817). $T\beta_4$ was prepared by solid phase peptide synthesis.

### Methods

### CF Sputum Viscosity Assay

For measuring the difference in viscosity between the samples incubated with $T\beta_4$ and water an apparatus was utilized that was used in a sliding assay which measured a rate of migration of sputum samples that were treated with varying amounts of $T\beta_4$ and corresponding water controls. The apparatus was a grooved plastic surface that could lie in a flat position and upon addition of samples be turned upright at a right angle and the sliding of the sample was measured (a modified tube gel casting stand). The surface was coated with silicon-spray to compensate for any variations of the surface of the apparatus. The migration distance of the apparatus was 6.9 cm.

Each sample contained 100 ug of sputum. The sputum was spread on a plate and the 10 ug samples were cut and weighed and placed in a siliconized

eppendorf tube. For each sample varying amounts of $T\beta_4$, as indicated below, was added to the 100 ug of sputum. For each $T\beta_4$ sample a corresponding control was done that contains an equal volume of water that was added to the $T\beta_4$ sample. Samples were incubated for 1 hour at 37°C. Samples were then placed on the apparatus and the migration distance was measured for 3 min. and a migration rate then calculated in mm/sec.

DNaseI Assay with $T\beta_4$

In defining the relationship between $T\beta_4$ and actin, an assay was used that utilizes the ability G-actin to bind to and inhibit DNaseI, in a one to one stoichiometric fashion. This relationship was used to indirectly measure the presence of G-actin in solution. The assay was a simple spectrophotometric assay that measured a relative change in absorbance at 260 nm. DNaseI will digest DNA in solution causing an increase in absorbance at 260 nm. G-actin inhibits this digestion, therefore inhibiting the change in absorbance at 260 nm.

Buffers

DNA Buffer - 0.1 M Tris/4 mM $MgSO_4$/1.8 mM $CaCl_2$.pH 7.5.

DNaseI Buffer - 50 mM Tris/10 $\mu$M PMSF/100 mM $CaCl_2$.pH 7.5.

G-Actin Buffer - 2mM Tris/0.5 mM DTT/0.5 mM ATP/0.2 mM $CaCl_2$/0.01% $NaN_3$

Polymerization buffer - 75 mM Immidazole/0.3 m KCl/6 mM $MgCl_2$

The assay was standardized before it was used for experiments with samples. Several tests were done using various amounts of DNaseI and DNA to define the assay system. The optimal conditions were defined for the DNaseI assay using the following method. DNA (Sigma Chemical Co. from bovine) was diluted to an absorbance at 260 nm of approximately 0.9 (0.1 mg/ml). DNaseI (Sigma Chemical) was diluted to absorbance of 0.04-0.06 units/min. (4-5 ul of 1 mg/ml). 10 $\mu$M of G-actin (in most samples) was incubated with 1/3 volume of polymerization buffer to polymerize the G-actin. 10 ul of polymerized actin

(F-actin) was incubated with varying amounts of $T\beta_4$ protein. $T\beta_4$ was allowed to incubate with F-actin for 1 hour at room temperature. After each of the sample incubations, 10 ul of DNaseI was added to a quartz cuvette and the actin/$T\beta_4$ sample was added to the cuvette containing the DNaseI. This was allowed to incubate at room temperature for 10 minutes. Then 1 ml of DNA was added to the cuvette and the absorbance at 260 nm was measured every 30 seconds for 3 minutes.

Results and Conclusions

Sputum migration assays

Fig. 2A represents a typical experiment with the CF sputum. From this data it can be seen that $T\beta_4$ significantly decreased the viscosity at doses 20 ug, 40 ug, and 100 ug. The migration rate measurements at higher volumes (the 150 ug measurement) tended to skew results because of the volume of liquid added to the samples. In samples that had volume increases of over 10% of total volume, the water added decreased the viscosity of the sample. The measurement at the 60 ug sample occurred approximately every 10 samples. In this case the water control slid faster than the treated sample. This was due to the thickness of the sputum before incubation; not every sputum sample was the same density.

Despite a few inconsistencies, the $T\beta_4$ had a significant effect on the sputum samples. This preliminary data was also supported by the following data from the in vitro DNaseI assay with $T\beta_4$ and F-actin. The results can be seen in Fig. 2A.

From the Fig. 3A data it can be seen that with an increase of $T\beta_4$ there was a decreased percentage of F-actin in the sample. This was seen in a decreased activity of DNaseI. This data demonstrates the ability of $T\beta_4$ to depolymerize actin filaments. Without being bound to any particular theory, this depolymerization activity may be due to $T\beta_4$ sequestering G-actin monomers, or $T\beta_4$ may bind directly to the filament and cause its depolymerization.

Actin monomers (i.e., G-actin) are released into the blood in large quantities in certain disease conditions when there is acute tissue injury or chronic infection. The strong tendency of G-actin to polymerize into long strands of F-actin fibrils in the blood quickly overwhelms the blood's "actin sequestering" system, clogging small capillaries in the lungs and elsewhere. $T\beta_4$ and $T\beta_4$ analogs, homologues and fragments are active in the blood where they prevent G-actin from forming capillary-clogging F-actin filaments.

In the lungs, in patients with chronic respiratory distress syndromes, the changes in the microvascular capillaries due to excess F-actin and actin complexes results in severe lung injury and inflammation. In diseases such as acute respiratory distress syndrome (ARDS), this microvascular pathology may be due to activation of the inflammatory cascade, particularly by by-products of bacterial infections such as endotoxin. Inhibitors of the inflammatory cytokines IL-1 or TNF do not appear to significantly improve survival or long-term outcome. Cell death, with the release of actin and the polymerization of G-actin into long fibrils that produce microangiopathy, leads not only to vascular occlusion, but to activation of complement, and elaboration of a large number of inflammatory mediators. $T\beta_4$ treatment is directed to the role that actin, and particularly actin polymerization, plays *in situ* in patients with cystic fibrosis, asthma, or other pulmonary diseases, who have increased turnover of airway cells associated with chronic airway inflammation and regeneration.

Without being bound to any particular theory, it is believed that the physiological conditions necessary for actin polymerization exist in the airway, and further that the $T\beta_4$ actin-scavenging and sequestering system also operates across the blood-airway barrier. In the laboratory, $T\beta_4$ has been shown to significantly reduce the toxicity and severity of septic shock and endotoxin-induced death in rodent experiments, and to down-regulate a number of cytokines associated with inflammatory diseases such as IL-1, IL-6, TNF-$\alpha$, and PAF. $T\beta_4$ can also down-regulate a number of other inflammatory molecules such as arachidonic acid metabolites, aldehydes, and free-radicals within the cell, and up-regulate glutathione.

According to the invention, $T\beta_4$ is believed to be effective in treating the acute and chronic lung diseases identified above, both by reducing the severity of actin toxicity in the blood (by maintaining actin in its sequestered G-actin form), and by down-regulating a number of cytokines, prostaglandin intermediates, and free radicals, which in excess are toxic and cause significant inflammation and accumulation of monocytes, neutrophils, and other cells that exacerbate tissue destruction. In preferred embodiments, $T\beta_4$ is administered by injection or by spraying $T\beta_4$ directly into the lungs.

$T\beta_4$ and $T\beta_4$ analogs, homologues and fragments having $T\beta_4$ activity appear to have the ability to both sequester actin monomers (G-actin), and down-regulate the major inflammatory cytokines such as IL-1$\alpha$, IL-6, TNF-$\alpha$, and PAF; as well as a number of arachidonic acid metabolites such as Tx$\beta_2$ and 6-keto-PGF1$\alpha$; in addition to lipid peroxidation. The cascade of free radicals and inflammatory molecules is deleterious, and contributes to the pathology of the lung diseases described above.

$T\beta_4$, when sprayed directly into the lungs, reduces inflammation and promotes healing by down-regulating the monocytes, neutrophils, and other white blood cells that exacerbate the inflammatory process. Given intravenously or by subcutaneous or intramuscular injection, $T\beta_4$ and $T\beta_4$ analogs, homologues and fragments reduce the clogging of lung capillaries and thus prevent death and promote healing by down-regulating the inflammatory cytokines and molecules produced during this process.

## Example 2

The objective was to determine the effects of $T\beta_4$ and DNase I on the properties of OAD sputum collected from six patients with stable CF lung disease.

Synthetic $T\beta_4$ was provided by Alpha 1 Biomedicals, Inc. and recombinant human DNase I (Pulmozyme®) was obtained from Genentech.

OAD sputum was analyzed untreated, and after the addition of amphibian Ringer's solution (negative control) mixed 1:5 v/v with the sputum,

as well as when treated with rhDNase I at 30 mcg/ml, $T\beta_4$ at 0.3, 3, 30 and 150 mcg/ml, or rhDNase I combined with $T\beta_4$ at 0.3 mcg/ml each. All specimens were incubated at 37°C for 30 minutes. Measurements were taken using a magnetic microrheometer. As described in King and Rubin, "Rheology of Airway Mucous" in *Airway Secretion*, Publisher Marcel Dekker, Inc., New York, Editors Takishima and Shimura, pages 283-314 (1994), the magnetic microrheometer measures viscoelastic properties of very small quantities of sputum. A steel ball was positioned in a 1-5 mcl sample of sputum and oscillated by an electromagnet at two different driving frequencies. The magnitude of displacement of the ball and its phase lag relative to the driving force were used to calculate the viscoelasticity of the sputum. The parameters measured were as follows:

G´ is storage modulus (elasticity) measured in dynes/cm$^2$;

G˝ is loss modulus in dynes/cm$^2$; loss modulus multiplied by rotational velocity corresponds to viscosity;

G* is mechanical impedance within a sputum sample, measured in dynes/cm$^2$, and is the vectorial sum of G´ and G˝;

Gs* is surface mechanical impedance, *i.e.*, mechanical impedance measured on the surface of a sputum sample in dynes/cm$^2$, and is the vectorial sum of G´ and G˝; and

tan $\delta$ is a ratio of G˝/G´.

For each sample, measurements were taken utilizing the magnetic microrheometer described in King and Rubin, *supra*, at a driving frequency of 1 radian/sec. (*e.g.*, G´ 1) corresponding to normal ciliary beats, and 100 radian/sec. (*e.g.*, G´ 100) corresponding to clearance by cough.

The results are shown in Tables 1-10 below and Figures 2B, 3B and 4-9, wherein "Groups" represent tested concentrations of rhDNase and/or $T\beta_4$, "cell" is a test cell in which a drug "Group" is tested, "cell mean" represents the mean average for a particular test "cell" in the units set forth above, and "count" represents sputum from the number of individuals tested. The Ringer's solution was 98.3 mmols/l NaCl, 2.7 mmols/l KCl, and 1.5 mmols/l CaCl.

Data analyses were performed using a StatView™ 4 statistics package (Abacus Concepts, Inc., Berkeley, CA) and a Power PC Macintosh® computer. The results demonstrate a synergistic effect with the combination of $T\beta_4$ and DNase I.

Without being bound to any particular theory, the synergistic effect on viscoelasticity brought about with a combination of $T\beta_4$ and DNase I may be explained by an enhanced effect of depolymerizing F-actin along with severing DNA. As DNase I also binds G-actin, which in turn inactivates DNase I activity, this synergy may also be due to enhanced DNase I activity by blocking the formation of actin-DNase I complexes.

## Table 1

Means Table for G' 1
Effect: Groups

| | Count | Mean | Std. Dev. | Std. Err. |
|---|---|---|---|---|
| Ringer's Control | 6 | 311.914 | 252.136 | 102.934 |
| rhDNase 30 mcg/ml | 6 | 200.795 | 132.383 | 54.037 |
| TB4 0.3 mcg/ml | 6 | 251.448 | 183.113 | 74.755 |
| TB4 3 mcg/ml | 6 | 245.405 | 234.730 | 95.828 |
| TB4 30 mcg/ml | 6 | 214.738 | 273.349 | 111.594 |
| TB4 150 mcg/ml | 6 | 199.139 | 284.766 | 116.255 |
| DNase + TB4 3+3 mcg/ml | 6 | 175.804 | 127.619 | 52.100 |

## Table 2

Means Table for G' 100
Effect: Groups

| | Count | Mean | Std. Dev. | Std. Err. |
|---|---|---|---|---|
| Ringer's Control | 6 | 879.517 | 514.096 | 209.879 |
| rhDNase 30 mcg/ml | 6 | 430.541 | 343.686 | 140.309 |
| TB4 0.3 mcg/ml | 6 | 572.524 | 363.184 | 148.289 |
| TB4 3 mcg/ml | 6 | 425.602 | 398.327 | 162.616 |
| TB4 30 mcg/ml | 6 | 385.463 | 410.186 | 167.458 |
| TB4 150 mcg/ml | 6 | 402.800 | 532.112 | 217.234 |
| DNase + TB4 3+3 mcg/ml | 6 | 290.601 | 306.929 | 125.303 |

## Table 3

Means Table for G" 1
Effect: Groups

| | Count | Mean | Std. Dev. | Std. Err. |
|---|---|---|---|---|
| Ringer's Control | 6 | 115.625 | 112.534 | 45.942 |
| rhDNase 30 mcg/ml | 6 | 70.110 | 28.761 | 11.742 |
| TB4 0.3 mcg/ml | 6 | 87.870 | 51.824 | 21.157 |
| TB4 3 mcg/ml | 6 | 87.087 | 83.053 | 33.908 |
| TB4 30 mcg/ml | 6 | 78.856 | 87.271 | 35.628 |
| TB4 150 mcg/ml | 6 | 51.885 | 62.694 | 25.595 |
| DNase + TB4 3+3 mcg/ml | 6 | 55.616 | 38.651 | 15.779 |

Table 4

Means Table for G" 100
Effect: Groupe

| | Count | Mean | Std. Dev. | Std. Err. |
|---|---|---|---|---|
| Ringer's Control | 6 | 316.898 | 254.448 | 103.878 |
| rhDNase 30 mcg/ml | 6 | 189.571 | 113.309 | 46.258 |
| TB4 0.3 mcg/ml | 6 | 288.569 | 119.712 | 48.872 |
| TB4 3 mcg/ml | 6 | 188.528 | 162.733 | 66.435 |
| TB4 30 mcg/ml | 6 | 176.159 | 141.417 | 57.733 |
| TB4 150 mcg/ml | 6 | 158.890 | 141.441 | 57.743 |
| DNase + TB4 3+3 mcg/ml | 6 | 147.064 | 135.730 | 55.411 |

Table 5

Means Table for G" 1
Effect: Groupe

| | Count | Mean | Std. Dev. | Std. Err. |
|---|---|---|---|---|
| Ringers Control | 6 | 332.372 | 272.312 | 111.171 |
| rhDNase 30 mcg/ml | 6 | 216.489 | 135.375 | 55.267 |
| TB4 0.3 mcg/ml | 6 | 268.308 | 189.511 | 77.368 |
| TB4 3 mcg/ml | 6 | 262.525 | 251.738 | 102.772 |
| TB4 30 mcg/ml | 6 | 228.752 | 283.664 | 115.805 |
| TB4 150 mcg/ml | 6 | 205.778 | 290.553 | 118.618 |
| DNase + TB4 3+3 mcg/ml | 6 | 165.150 | 142.101 | 58.013 |

Table 6

Means Table for G" 100
Effect: Groupe

| | Count | Mean | Std. Dev. | Std. Err. |
|---|---|---|---|---|
| Ringer's Control | 6 | 751.615 | 566.221 | 231.159 |
| rhDNase 30 mcg/ml | 6 | 470.548 | 360.673 | 147.244 |
| TB4 0.3 mcg/ml | 6 | 633.453 | 362.845 | 148.131 |
| TB4 3 mcg/ml | 6 | 464.464 | 427.055 | 174.344 |
| TB4 30 mcg/ml | 6 | 430.879 | 429.886 | 175.500 |
| TB4 150 mcg/ml | 6 | 438.765 | 550.637 | 224.796 |
| DNase + TB4 3+3 mcg/ml | 6 | 286.722 | 330.983 | 135.123 |

Table 7

Means Table for Gs* 1
Effect: Groups

| | Count | Mean | Std. Dev. | Std. Err. |
|---|---|---|---|---|
| Ringer's Control | 6 | 432.084 | 310.454 | 126.742 |
| rhDNase 30 mcg/ml | 6 | 200.528 | 129.489 | 52.864 |
| TB4 0.3 mcg/ml | 6 | 262.169 | 216.720 | 88.476 |
| TB4 3 mcg/ml | 6 | 209.635 | 155.661 | 63.548 |
| TB4 30 mcg/ml | 6 | 235.234 | 349.118 | 142.527 |
| TB4 150 mcg/ml | 6 | 212.894 | 316.804 | 129.335 |
| DNase + TB4 3+3 mcg/ml | 6 | 158.254 | 160.305 | 65.444 |

Table 8

Means Table for tan ∂ 1
Effect: Groups

| | Count | Mean | Std. Dev. | Std. Err. |
|---|---|---|---|---|
| Ringer's Control | 6 | 0.340 | 0.074 | 0.030 |
| rhDNase 30 mcg/ml | 6 | 0.392 | 0.101 | 0.041 |
| TB4 0.3 mcg/ml | 6 | 0.382 | 0.128 | 0.052 |
| TB4 3 mcg/ml | 6 | 0.343 | 0.074 | 0.030 |
| TB4 30 mcg/ml | 6 | 0.467 | 0.188 | 0.077 |
| TB4 150 mcg/ml | 6 | 0.305 | 0.068 | 0.028 |
| DNase + TB4 3+3 mcg/ml | 6 | 0.327 | 0.050 | 0.020 |

Table 9

Means Table for tan ∂ 100
Effect: Groups

| | Count | Mean | Std. Dev. | Std. Err. |
|---|---|---|---|---|
| Ringer's Control | 6 | 0.502 | 0.180 | 0.073 |
| rhDNase 30 mcg/ml | 6 | 0.500 | 0.116 | 0.047 |
| TB4 0.3 mcg/ml | 6 | 0.520 | 0.206 | 0.084 |
| TB4 3 mcg/ml | 6 | 0.463 | 0.056 | 0.023 |
| TB4 30 mcg/ml | 6 | 0.580 | 0.344 | 0.140 |
| TB4 150 mcg/ml | 6 | 0.485 | 0.151 | 0.062 |
| DNase + TB4 3+3 mcg/ml | 6 | 0.488 | 0.043 | 0.017 |

While the invention has been described and illustrated with details and references to certain preferred embodiments, those skilled in the art will appreciate that various modifications, changes, omissions, and substitutes can be made without departing from the spirit of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: David R. CROCKFORD, Bruce K. RUBIN, Michael L. BERMAN and Allan L. GOLDSTEIN

(ii) TITLE OF INVENTION: METHOD OF TREATING OBSTRUCTIVE AIRWAY DISEASE BY ADMINISTRATION OF THYMOSIN $\beta_4$, OR CO-ADMINISTRATION OF THYMOSIN $\beta_4$ AND DNase I

(iii) NUMBER OF SEQUENCES: 16

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Rothwell, Figg, Ernst & Kurz, pc
(B) STREET: Suite 701-E, 555-13th Street, N.W.
(C) CITY: Washington, D.C.
(E) COUNTRY: United States of America
(F) ZIP CODE: 20004

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5" High Density 3M Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: Microsoft® DOS Version 6.22
(D) SOFTWARE: WordPerfect® Version 5.1+

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION: Unknown

(vii) PRIOR APPLICATION DATA: None

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: George R. Repper
(B) REGISTRATION NUMBER: 31,414
(C) REFERENCE NUMBER: 1783-145PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (202) 783-6040
(B) TELEFAX: (202) 783-6031
(C) TELEX: (International) 64285

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

Ser Asp Lys Pro Asp Met Ala Glu Ile Glu Lys Phe Asp Lys Ser Lys
1 5 10 15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Pro Leu Pro Ser Lys Glu
20 25 30

Thr Ile Glu Gln Glu Lys Gln Ala Gly Glu Ser
35 40

(3) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 2:

Ser Asp Lys Pro
1

(4) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 3:

Lys Leu Lys Lys Thr Gly Thr
1 5

(5) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 4:

Lys Leu Lys Lys Thr Glu Thr Glu Thr Gly Glu Lys
1 5 10

(6) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 5:

Asp Lys Ser Lys Leu Lys Lys Thr Glu Thr Gly Glu Lys
1 5 10

(7) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 6:

Lys Ser Lys Leu Lys Lys Thr Glu Thr Gly Glu Lys
1 5 10

(8) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 7:

Glu Lys Phe Asp Lys Ser Lys Leu Lys Lys Thr Gly Thr Gly Glu Lys
1 5 10 15

Asn Pro Leu

(9) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 8:

```
Ala Asp Lys Pro Asp Met Ala Glu Ile Glu Lys Phe Asp Lys Ser Lys
1               5                  10                  15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Pro Leu Pro Ser Lys Glu
            20                  25                  30

Thr Ile Glu Gln Glu Lys Gln Ala Gly Glu Ser
        35                  40
```

(10) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 9:

```
Ser Asp Lys Pro Asp Met Ala Glu Ile Glu Lys Phe Asp Lys Ser Lys
1               5                  10                  15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Pro Leu Pro Ser Lys Glu
            20                  25                  30

Thr Ile Glu Gln Glu Lys Gln Ala Gly Glu Ser
        35                  40
```

(11) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 10:

Ala Asp Lys Pro Asp Leu Gly Glu Ile Asn Ser Phe Asp Lys Ala Lys
1 5 10 15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Thr Leu Pro Thr Lys Glu
20 25 30

Thr Ile Glu Gln Glu Lys Gln Ala Lys
35 40

(12) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 11:

Ala Asp Lys Pro Asp Met Gly Glu Ile Asn Ser Phe Asp Lys Ala Lys
1 5 10 15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Thr Leu Pro Thr Lys Glu
20 25 30

Thr Ile Glu Gln Glu Lys Gln Ala Lys
35 40

(13) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 12:

Ala Asp Lys Pro Asp Met Gly Glu Ile Ala Ser Phe Asp Lys Ala Lys
1 5 10 15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Thr Leu Pro Thr Lys Glu
20 25 30

Thr Ile Glu Gln Glu Lys Arg Ser Glu Ile Ser
35 40

(14) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 13:

```
Ser Asp Lys Pro Asn Leu Glu Glu Val Ala Ser Phe Asp Lys Thr Lys
1               5                  10                  15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Pro Leu Pro Thr Lys Glu
            20                  25                  30

Thr Ile Glu Gln Glu Lys Gln Ala Ser
        35                  40
```

(15) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 14:

```
Ser Asp Lys Pro Asp Leu Ala Glu Val Ser Asn Phe Asp Lys Thr Lys
1               5                  10                  15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Pro Leu Pro Thr Lys Glu
            20                  25                  30

Thr Ile Glu Gln Glu Lys Gln Ala Thr Ala
        35                  40
```

(16) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 15:

Ala Asp Lys Pro Asp Met Gly Glu Ile Ala Ser Phe Asp Lys Ala Lys
1 5 10 15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Thr Leu Pro Thr Lys Glu
20 25 30

Thr Ile Glu Gln Glu Lys Gln Ala Lys
35 40

(17) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 16:

Ser Asp Lys Pro Asp Ile Ser Glu Val Ser Ser Phe Asp Lys Thr Lys
1 5 10 15

Leu Lys Lys Thr Glu Thr Ala Glu Lys Asn Thr Leu Pro Thr Lys Glu
20 25 30

Thr Ile Glu Gln Glu Lys Thr Ala
35 40

What is claimed is:

1. A method of reducing viscoelasticity of sputum of obstructive airway disease (OAD), comprising contacting OAD sputum with a viscoelasticity-reducing amount of Thymosin $\beta_4$ ($T\beta_4$) or a combination of $T\beta_4$ and DNase I.

2. The method of claim 1, wherein said $T\beta_4$ or said combination of $T\beta_4$ and DNase I are present in a pharmaceutical formulation including a pharmaceutically-acceptable liquid carrier.

3. The method of claim 2, further including the step of administering the pharmaceutical formulation to an OAD patient by introducing said formulation into an airway of said patient, so as to contact said formulation with said sputum.

4. The method of claim 3, wherein said pharmaceutical formulation is in aerosol form.

5. The method of claim 4, wherein said $T\beta_4$ and said DNase I are present in said pharmaceutical formulation in a respective ratio of from about 1:2 to about 2:1.

6. The method of claim 4 or 5, wherein the concentration of said $T\beta_4$ in said formulation is within the range of from about 0.1 mcg/ml to about 10 mg/ml, or the concentrations of said $T\beta_4$ and said DNase I in said formulation are each within the range of from about 0.1 mcg/ml to about 10 mg/ml.

7. The method of claim 6, wherein said range is about 0.1-10 mg/ml.

8. The method of claim 7, wherein said range is about 0.3-7 mg/ml.

9. The method of claim 8, wherein said formulation is administered to said patient so that about 0.5-10 mg/day of each of said $T\beta_4$ and said DNase I is administered to said patient.

10. The method of claim 9, wherein said ratio is about 1:1.

11. The method of claim 10, wherein about 2.5-5 mg/day of each of said $T\beta_4$ and DNase I is administered to said patient.

12. The method of claim 8 or 11, wherein said patient is a cystic fibrosis (CF) patient.

13. The method of claim 12, wherein each said concentration is within the range of about 0.1-200 mcg/ml.

14. The method of claim 12, wherein each said concentration is within the range of 0.1-10 mg/ml.

15. A pharmaceutical formulation for use in reducing viscoelasticity of sputum of OAD, comprising an OAD sputum viscoelasticity-reducing amount of $T\beta_4$, or a combination of $T\beta_4$ and DNase I.

16. The pharmaceutical formulation of claim 15, further including a pharmaceutically-acceptable liquid carrier, wherein $T\beta_4$ is at a concentration within the range of from about 0.1 mcg/ml to about 10 mg/ml, or wherein said $T\beta_4$ and said DNase I in said combination each have a concentration within the range of from about 0.1 mcg/ml to about 10 mg/ml, and said $T\beta_4$ and said DNase I in said combination are present in a respective ratio of about 1:2 to about 2:1.

17. The pharmaceutical formulation of claim 16, wherein said range is about 0.3-7 mg/ml.

18. The pharmaceutical formulation of claim 17, wherein said range is about 0.5-5 mg/ml.

19. The pharmaceutical formulation of claim 16, wherein said range is about 0.1-10 mg/ml, said ratio is about 1:1 and said pharmaceutical formulation is in aerosol form.

20. The pharmaceutical formulation of claim 19, wherein said range is about 0.5-5 mg/ml.

Amino Acid Sequences of thymosin $\beta_4$ homologous T$\beta_4$ proteins and T$\beta$4 fragments

Thymosin $\beta_4$

Ac-Ser-Asp-Lys-Pro-Asp-Met-Ala-Glu-Ile-Glu Lys-Phe-Asp-Lys-Ser-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-Glu-Lys-Asn-Pro-Leu-Pro-Ser-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Gly-Glu-Ser-OH

[$N_{1-4}$]-Thymosin $\beta_4$

Ac-Ser-Asp-Lys-Pro

[$N_{16-22}$]-Thymosin $\beta_4$

Lys-Leu-Lys-Lys-Thr-Glu-Thr

[$N_{16-24}$]-Thymosin $\beta_4$

Lys-Leu-Lys-Lys-Thr-Glu-Thr-Glu-Thr-Gly-GLu-Lys

[$N_{13-25}$]-Thymosin $\beta_4$

Asp-Lys-Ser-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gly-Glu-Lys

[$N_{14-25}$]-Thymosin $\beta_4$

Lys-Ser-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gly-Glu-Lys

[$N_{16-28}$]-Thymosin $\beta_4$

Glu-Lys-Phe-Asp-Lys-Ser-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gly-GLu-Lys-Asn-Pro-Leu

FIG.1A

Thymosin $\beta_4$*Ala

Ac-Ala-Asp-Lys-Pro-Asp-Met-Ala-Glu-Ile-Glu-Lys-Phe-Asp-Lys-Ser-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-
Glu-Lys-Asn-Pro-Leu-Pro-Ser-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Gly-Glu-Ser-OH

Thymosin $\beta_4$-Xen

Ac-Ser-Asp-Lys-Pro-Asp-Met-Ala-Glu-Ile-Glu-Lys-Phe-Asp-Lys-Ala-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-
Glu-Lys-Asn-Pro-Leu-Pro-Ser-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Thr-Ser-Glu-Ser-OH

Thymosin $\beta_9$

Ac-Ala-Asp-Lys-Pro-Asp-Leu-Gly-Glu-Ile-Asn-Ser-Phe-Asp-Lys-Ala-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-
Glu-Lys-Asn-Thr-Leu-Pro-Thr-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Lys-OH

Thymosin $\beta_9$-MET

Ac-Ala-Asp-Lys-Pro-Asp-Met-Gly-Glu-Ile-Asn-Ser-Phe-Asp-Lys-Ala-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-
Glu-Lys-Asn-Thr-Leu-Pro-Thr-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Lys-OH

Thymosin $\beta_{10}$

Ac-Ala-Asp-Lys-Pro-Asp-Met-Gly-Glu-Ile-Ala-Ser-Phe-Asp-Lys-Ala-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-
Glu-Lys-Asn-Thr-Leu-Pro-Thr-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Arg-Ser-Glu-Ile-Ser-OH

FIG.1B

Thymosin $\beta_{11}$

Ac-Ser-Asp-Lys-Pro-Asn-Leu-Glu-Glu-Val-Ala-Ser-Phe-Asp-Lys-Thr-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-
Glu-Lys-Asn-Pro-Leu-Pro-Thr-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Ser-OH

Thymosin $\beta_{12}$

Ac-Ser-Asp-Lys-Pro-Asp-Leu-Ala-Glu-Val-Ser-Asn-Phe-Asp-Lys-Thr-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-
Glu-Lys-Asn-Pro-Leu-Pro-Thr-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Thr-Ala-OH

Thymosin $\beta_{13}$

Ac-Ala-Asp-Lys-Pro-Asp-Met-Gly-Glu-Ile-Ala-Ser-Phe-Asp-Lys-Ala-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-
Glu-Lys-Asn-Thr-Leu-Pro-Thr-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Lys-OH

Thymosin $\beta_{14}$

Ac-Ser-Asp-Lys-Pro-Asp-Ile-Ser-Glu-Val-Ser-Ser-Phe-Asp-Lys-Thr-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Ala-
Glu-Lys-Asn-Thr-Leu-Pro-Thr-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Thr-Ala-OH

FIG.1C

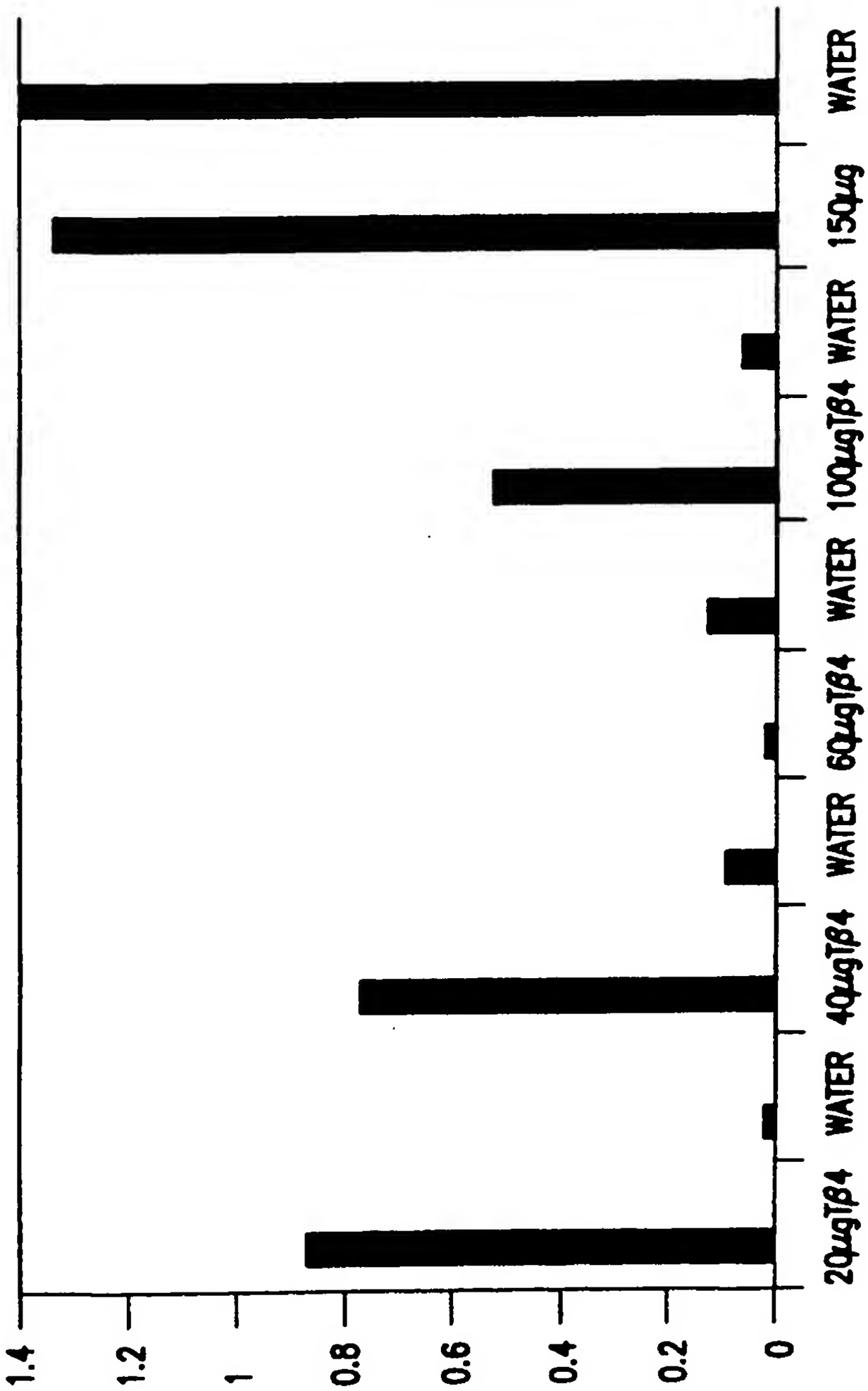
20μgT84
WATER
40μgT84
WATER
60μgT84
WATER
100μgT84
WATER
150μg
WATER
0
0.2
0.4
0.6
0.8
1
1.2
1.4

FIG.2A

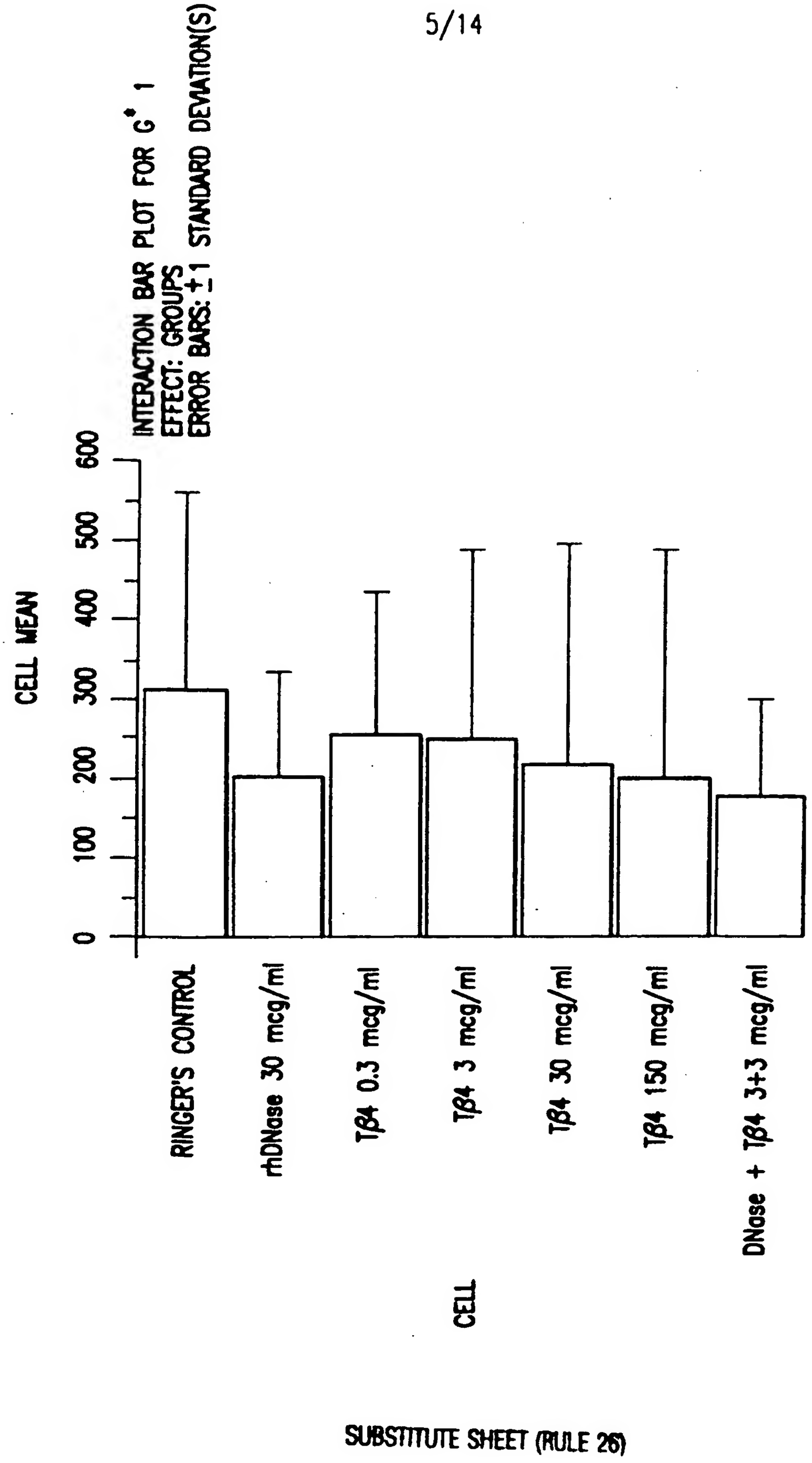
INTERACTION BAR PLOT FOR G' 1
EFFECT: GROUPS
ERROR BARS: +1 STANDARD DEVIATION(S)
CELL MEAN
0
100
200
300
400
500
600
CELL
RINGER'S CONTROL
rhDNase 30 mcg/ml
Tβ4 0.3 mcg/ml
Tβ4 3 mcg/ml
Tβ4 30 mcg/ml
Tβ4 150 mcg/ml
DNase + Tβ4 3+3 mcg/ml

FIG.2B

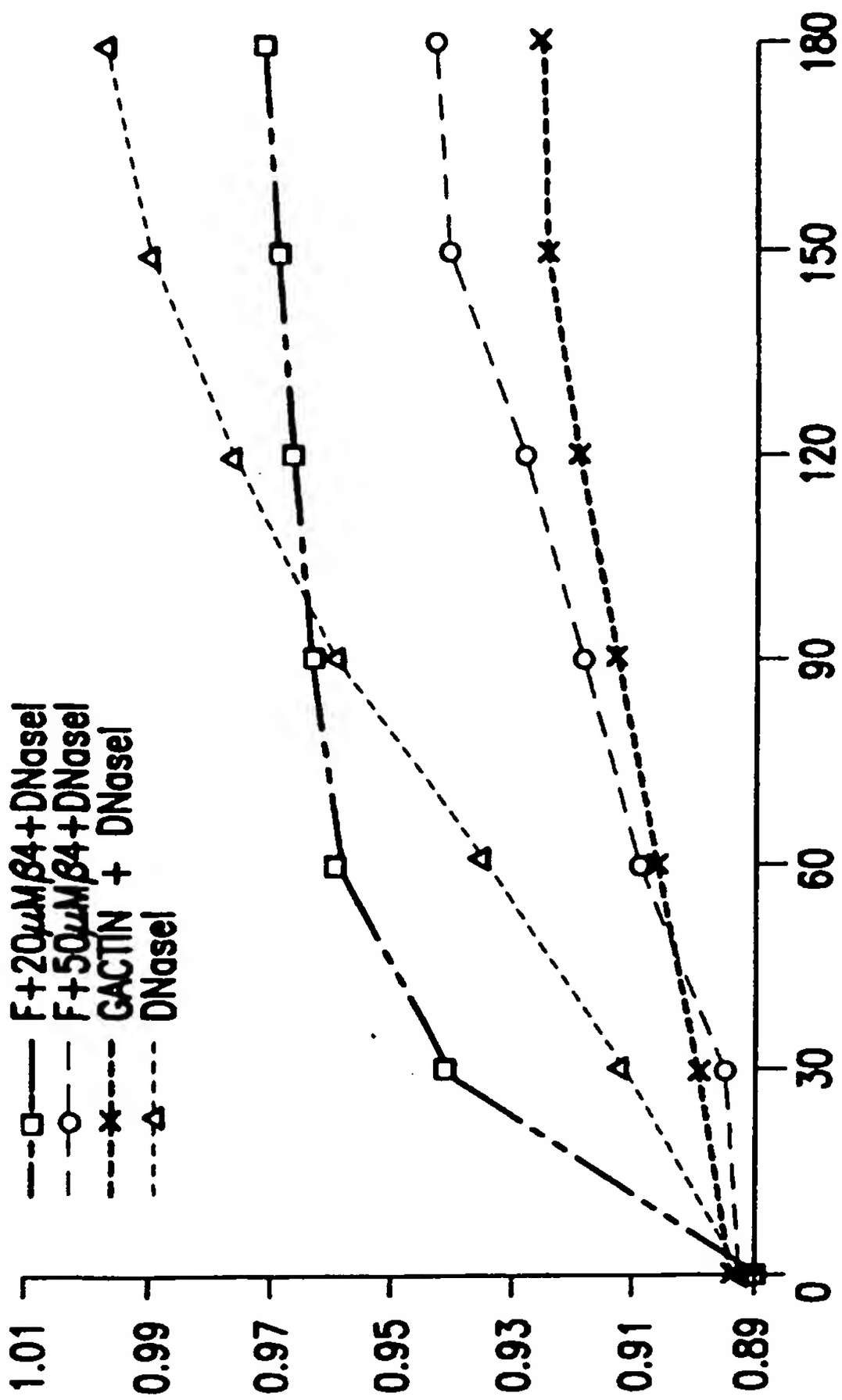
CHANGE IN Abs. @ 260nm
1.01
0.99
0.97
0.95
0.93
0.91
0.89
0
30
60
90
120
150
180
TIME IN SECONDS
F+20μMβ4+DNaseI
F+50μMβ4+DNaseI
GACTIN + DNaseI
DNaseI

FIG.3A

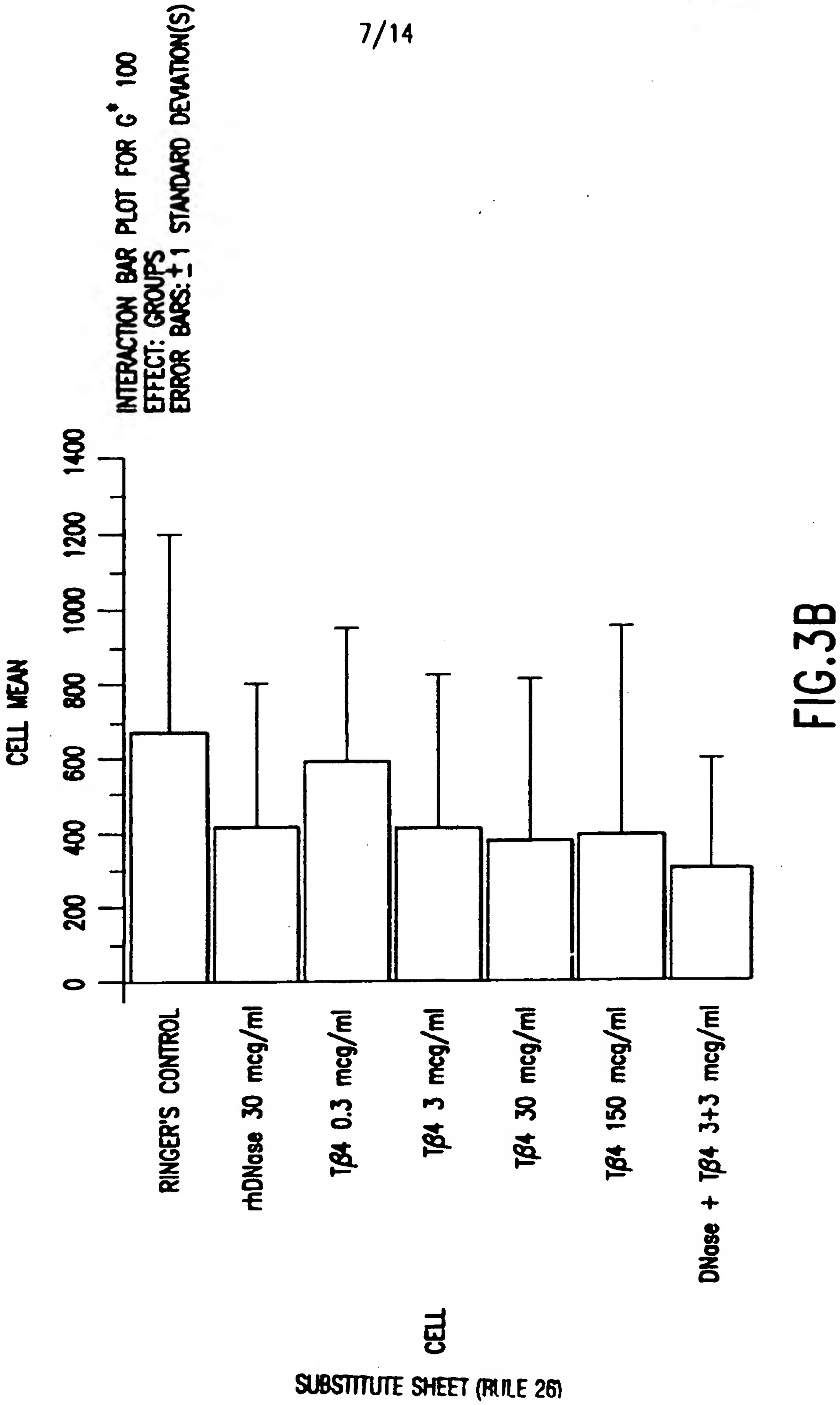
INTERACTION BAR PLOT FOR C* 100
EFFECT: GROUPS
ERROR BARS: +-1 STANDARD DEVIATION(S)
CELL MEAN
0
200
400
600
800
1000
1200
1400
RINGER'S CONTROL
rhDNase 30 mcg/ml
Tβ4 0.3 mcg/ml
Tβ4 3 mcg/ml
Tβ4 30 mcg/ml
Tβ4 150 mcg/ml
DNase + Tβ4 3+3 mcg/ml
CELL

FIG.3B

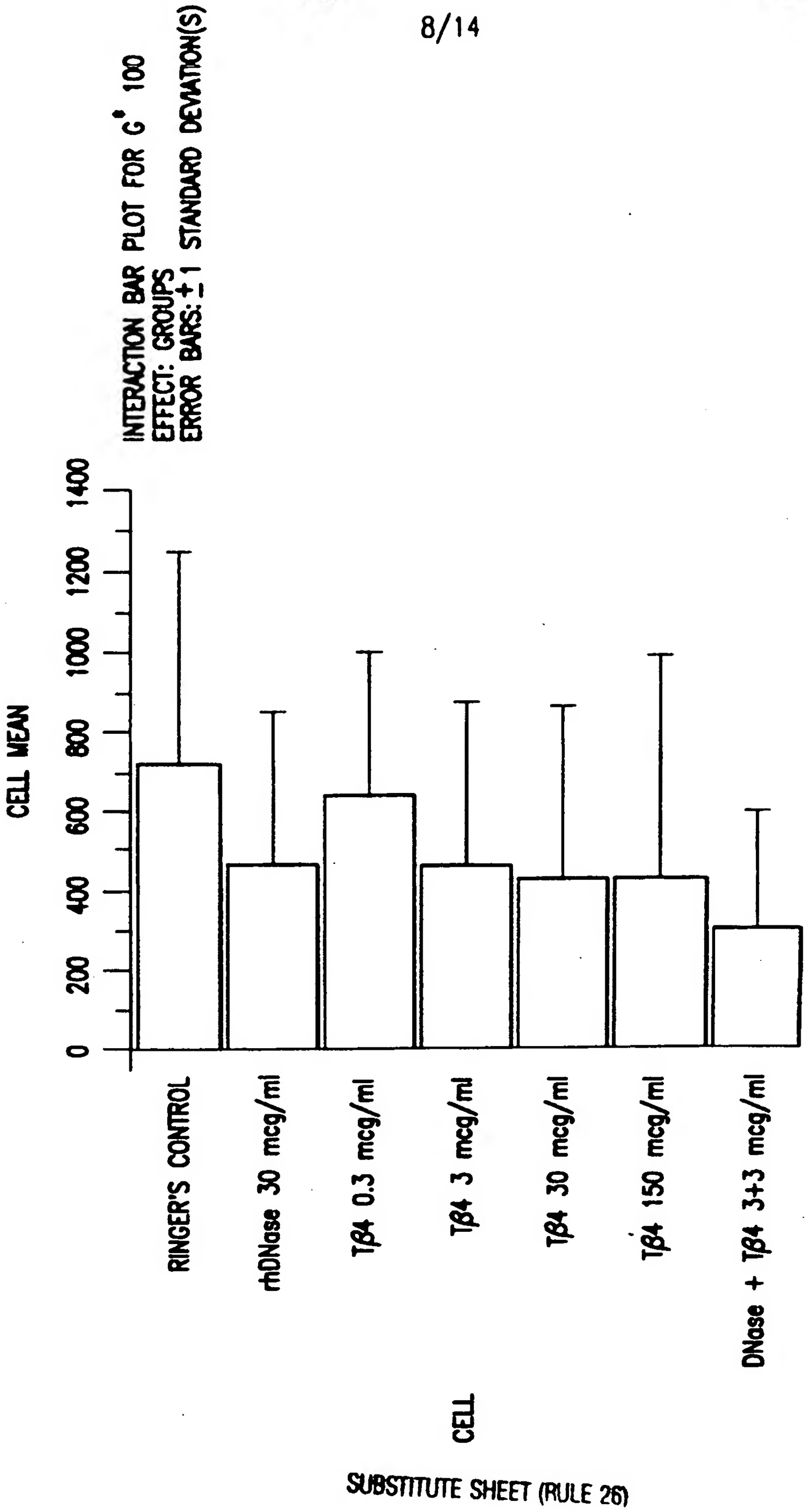
INTERACTION BAR PLOT FOR C' 100
EFFECT: GROUPS
ERROR BARS: +1 STANDARD DEVIATION(S)
CELL MEAN
0
200
400
600
800
1000
1200
1400
RINGER'S CONTROL
rhDNase 30 mcg/ml
Tβ4 0.3 mcg/ml
Tβ4 3 mcg/ml
Tβ4 30 mcg/ml
Tβ4 150 mcg/ml
DNase + Tβ4 3+3 mcg/ml
CELL

FIG.4

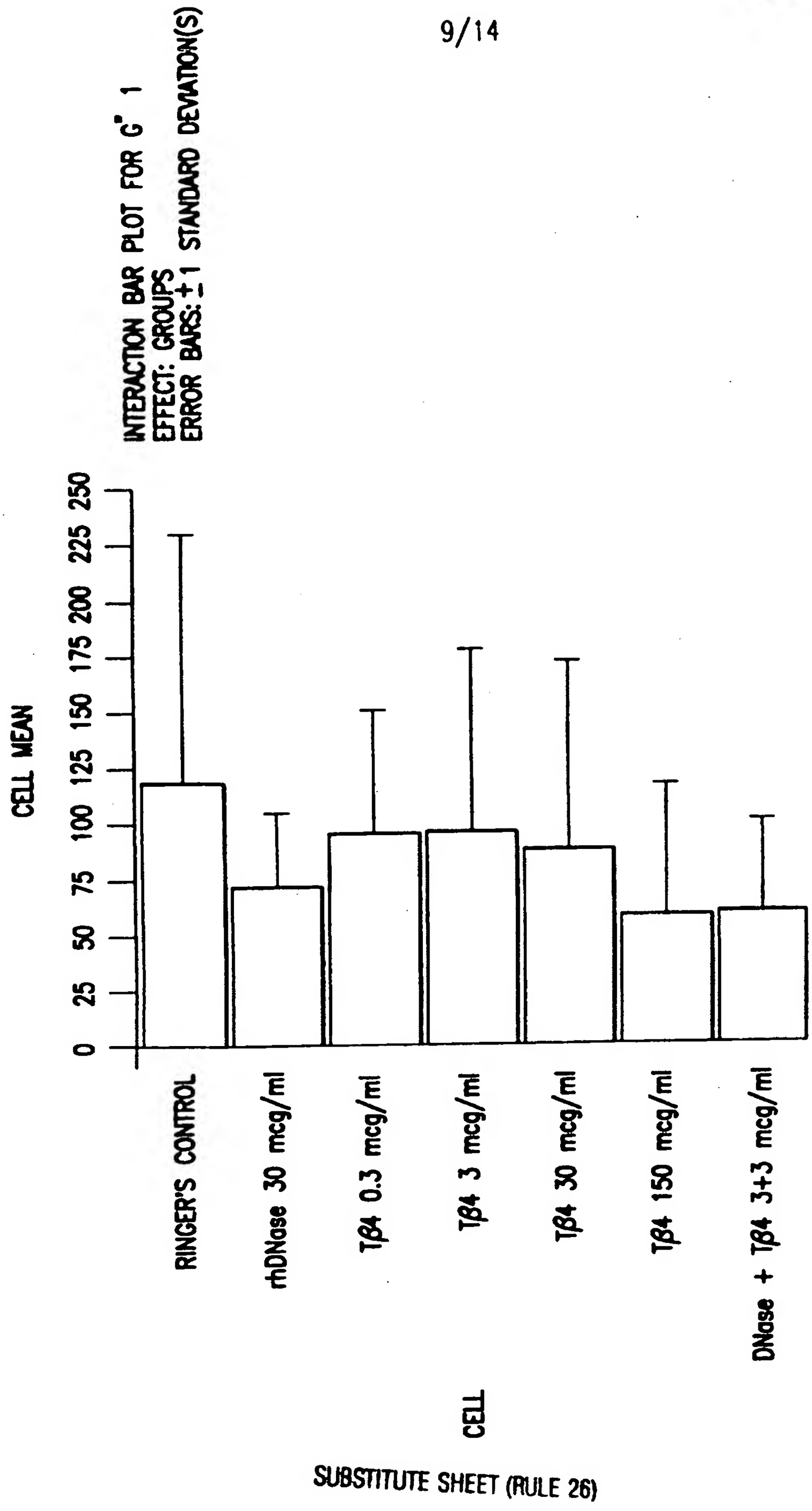
INTERACTION BAR PLOT FOR G' 1
EFFECT: GROUPS
ERROR BARS: +-1 STANDARD DEVIATION(S)
CELL MEAN
0
25
50
75
100
125
150
175
200
225
250
RINGER'S CONTROL
rhDNase 30 mcg/ml
Tβ4 0.3 mcg/ml
Tβ4 3 mcg/ml
Tβ4 30 mcg/ml
Tβ4 150 mcg/ml
DNase + Tβ4 3+3 mcg/ml
CELL

FIG.5

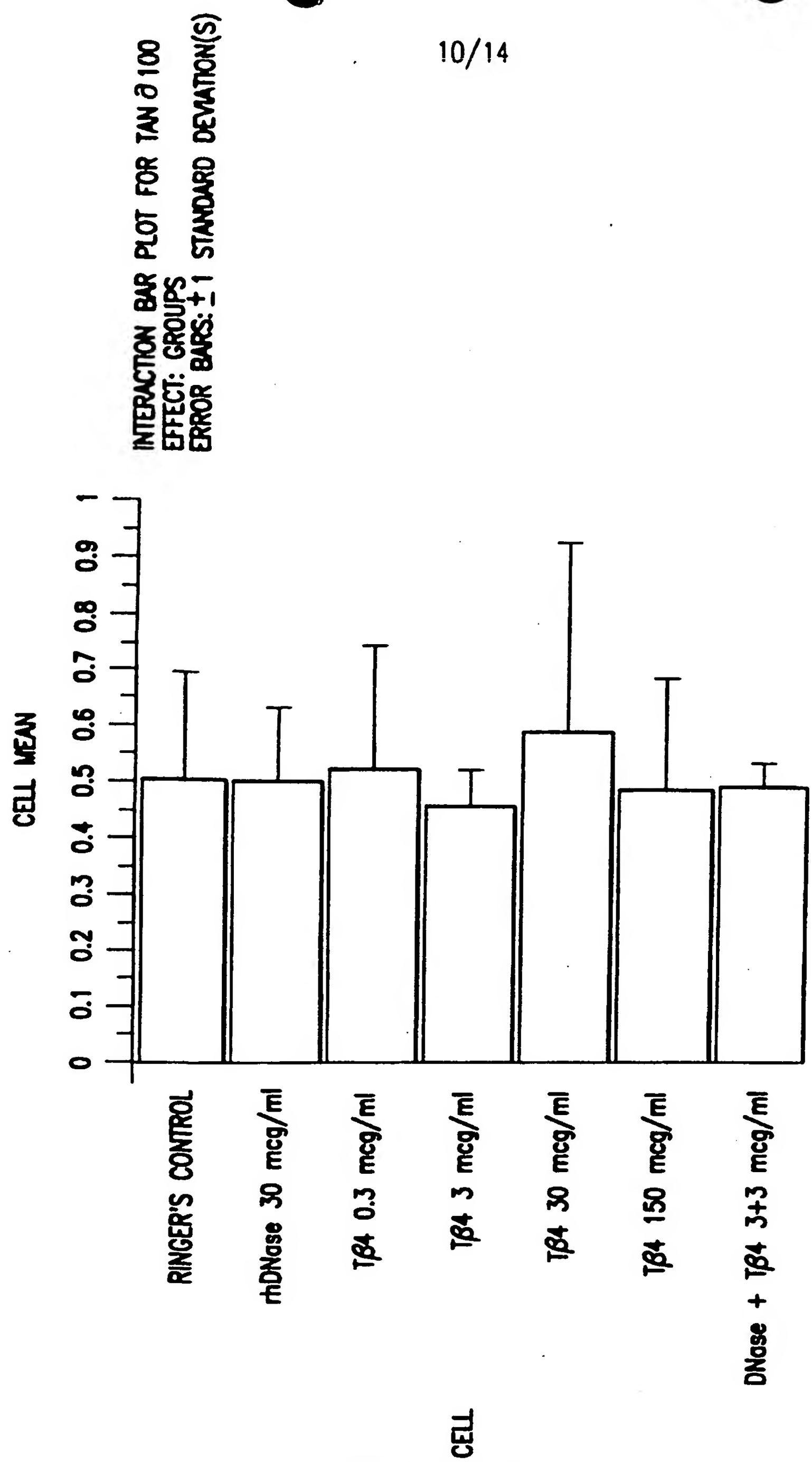
INTERACTION BAR PLOT FOR TAN θ 100
EFFECT: GROUPS
ERROR BARS: +-1 STANDARD DEVIATION(S)
CELL MEAN
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
RINGER'S CONTROL
rhDNase 30 mcg/ml
Tβ4 0.3 mcg/ml
Tβ4 3 mcg/ml
Tβ4 30 mcg/ml
Tβ4 150 mcg/ml
DNase + Tβ4 3+3 mcg/ml
CELL

FIG.6

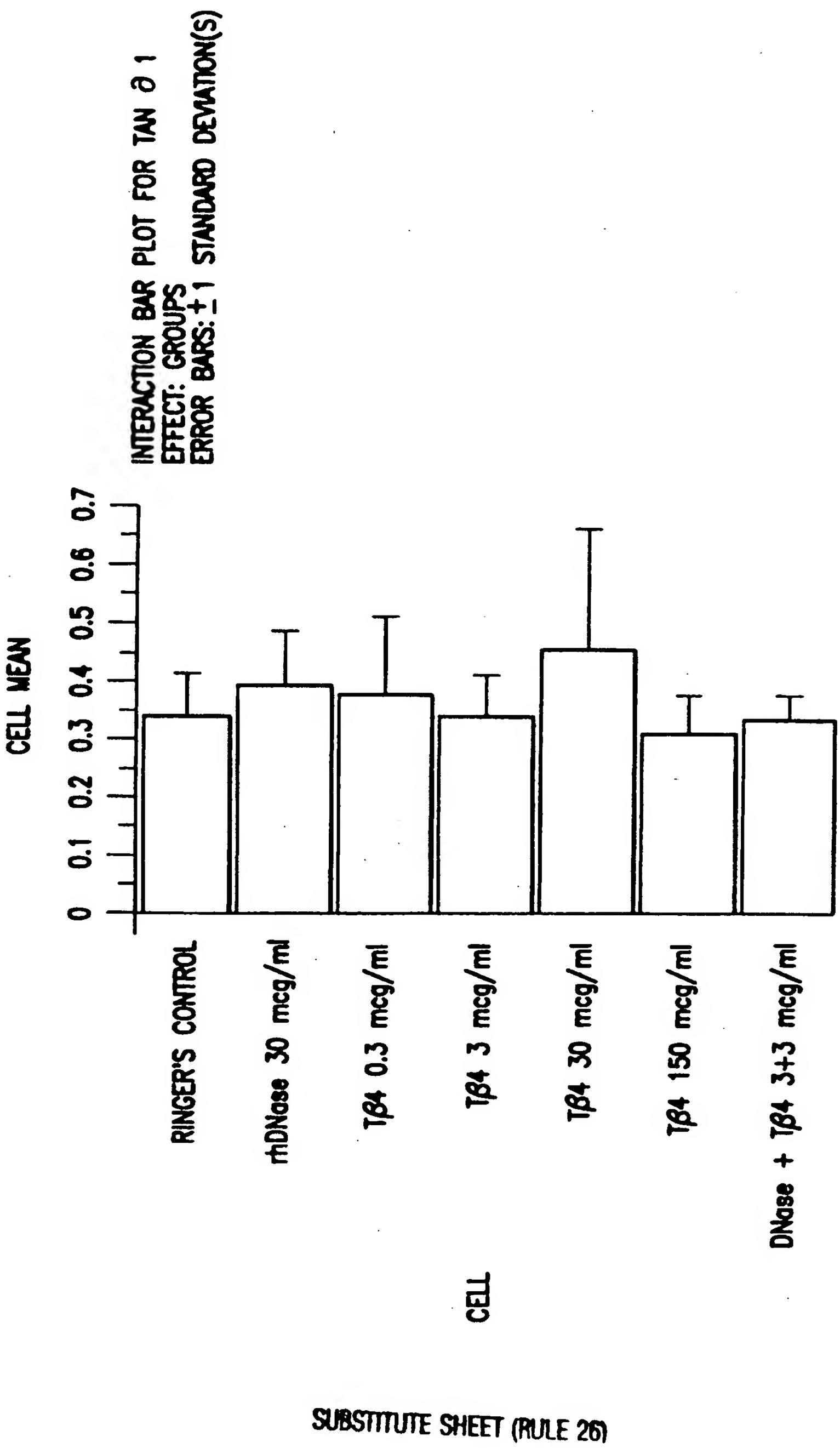
PLOT FOR TAN ∂ 1
STANDARD DEVIATION(S)
INTERACTION BAR
EFFECT: GROUPS
ERROR BARS: +1
CELL MEAN
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
RINGER'S CONTROL
rhDNase 30 mcg/ml
Tβ4 0.3 mcg/ml
Tβ4 3 mcg/ml
Tβ4 30 mcg/ml
Tβ4 150 mcg/ml
DNase + Tβ4 3+3 mcg/ml
CELL

FIG.7

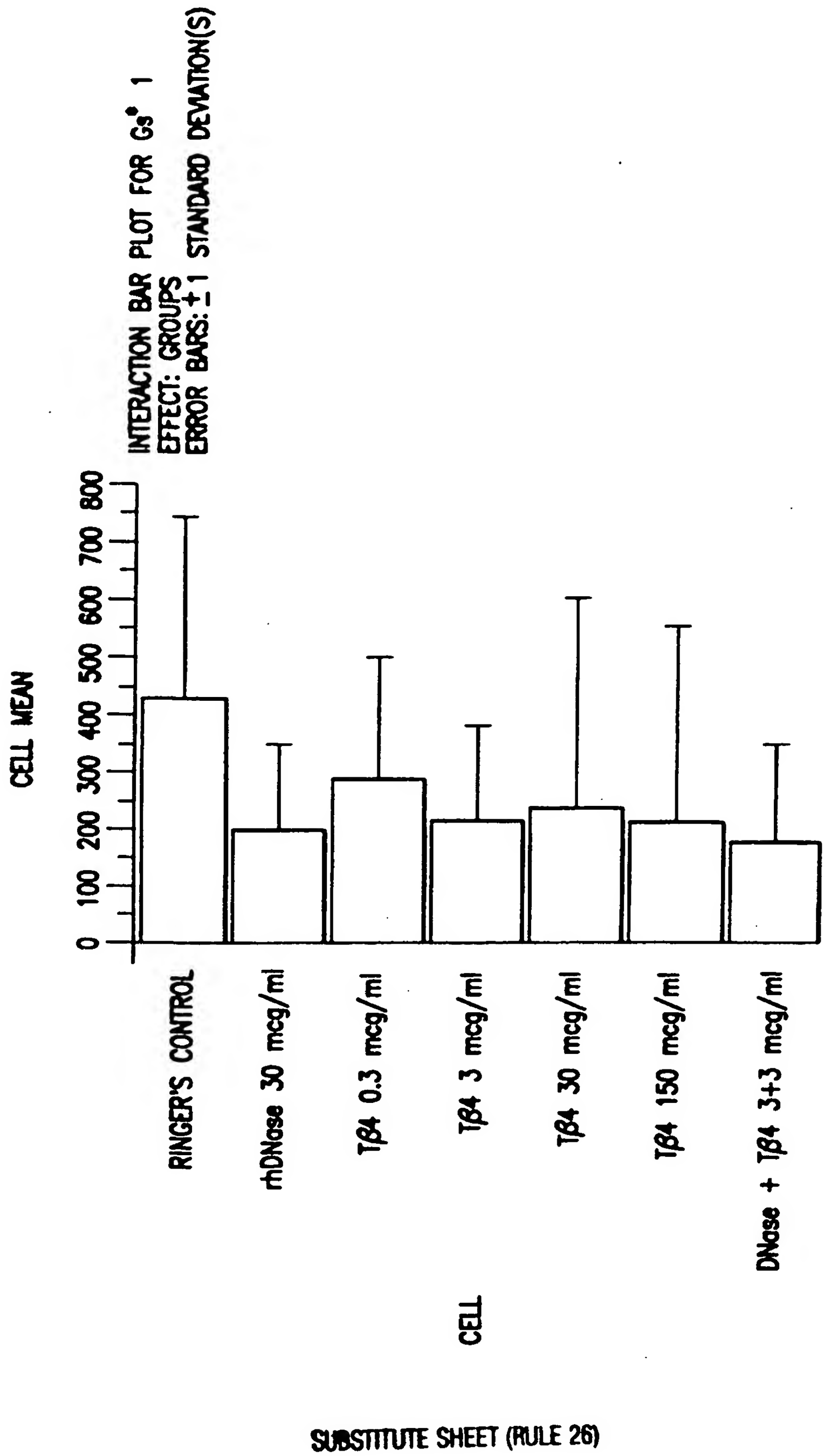
INTERACTION BAR PLOT FOR Gs* 1
EFFECT: GROUPS
ERROR BARS: +-1 STANDARD DEVIATION(S)
CELL MEAN
0 100 200 300 400 500 600 700 800
RINGER'S CONTROL
rhDNase 30 mcg/ml
Tβ4 0.3 mcg/ml
Tβ4 3 mcg/ml
Tβ4 30 mcg/ml
Tβ4 150 mcg/ml
DNase + Tβ4 3+3 mcg/ml
CELL

FIG.8

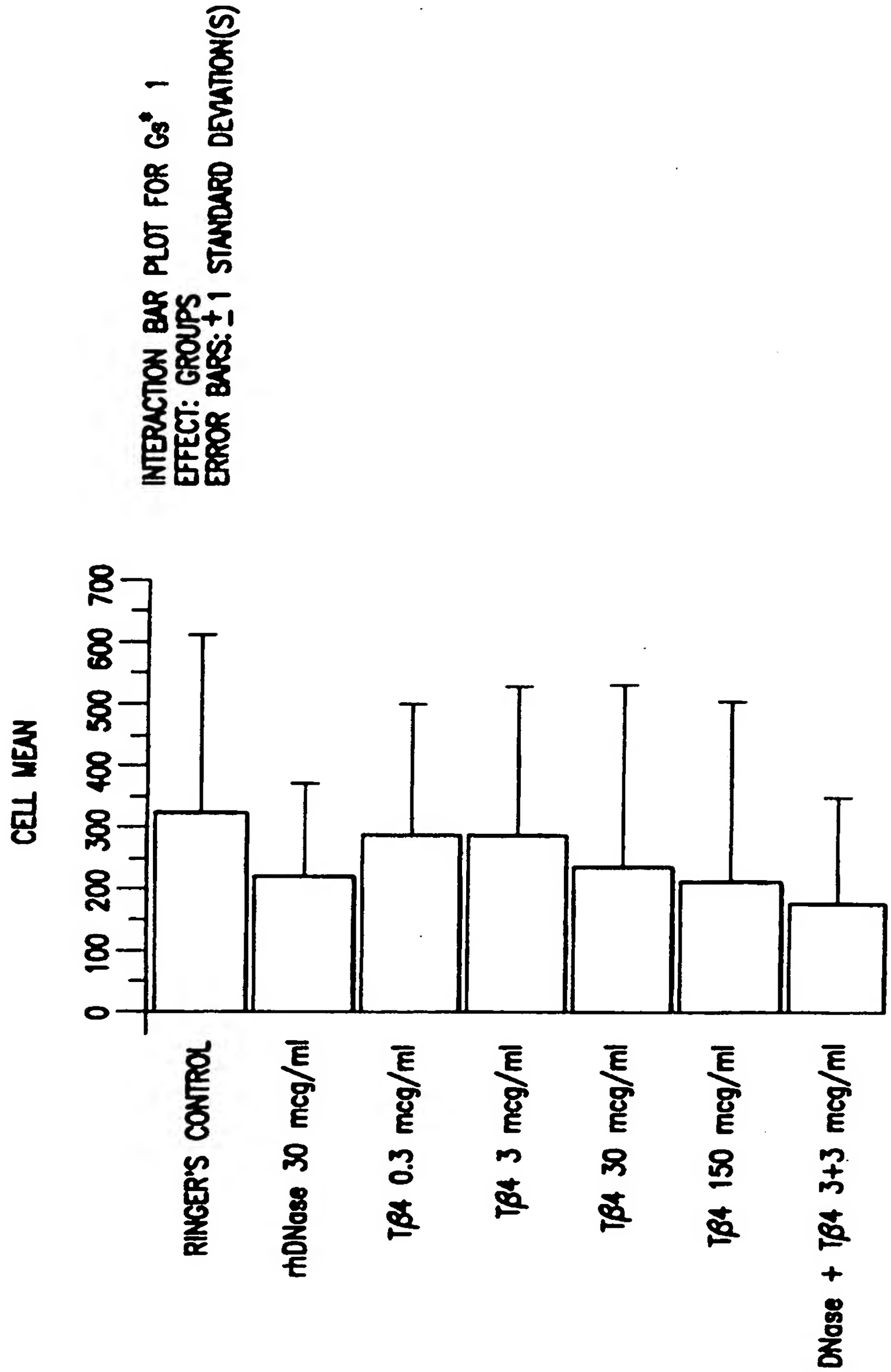
INTERACTION BAR PLOT FOR Cs* 1
EFFECT: GROUPS
ERROR BARS: +1 STANDARD DEVIATION(S)
CELL MEAN
0 100 200 300 400 500 600 700
RINGER'S CONTROL
rhDNase 30 mcg/ml
Tβ4 0.3 mcg/ml
Tβ4 3 mcg/ml
Tβ4 30 mcg/ml
Tβ4 150 mcg/ml
DNase + Tβ4 3+3 mcg/ml
CELL

FIG.9

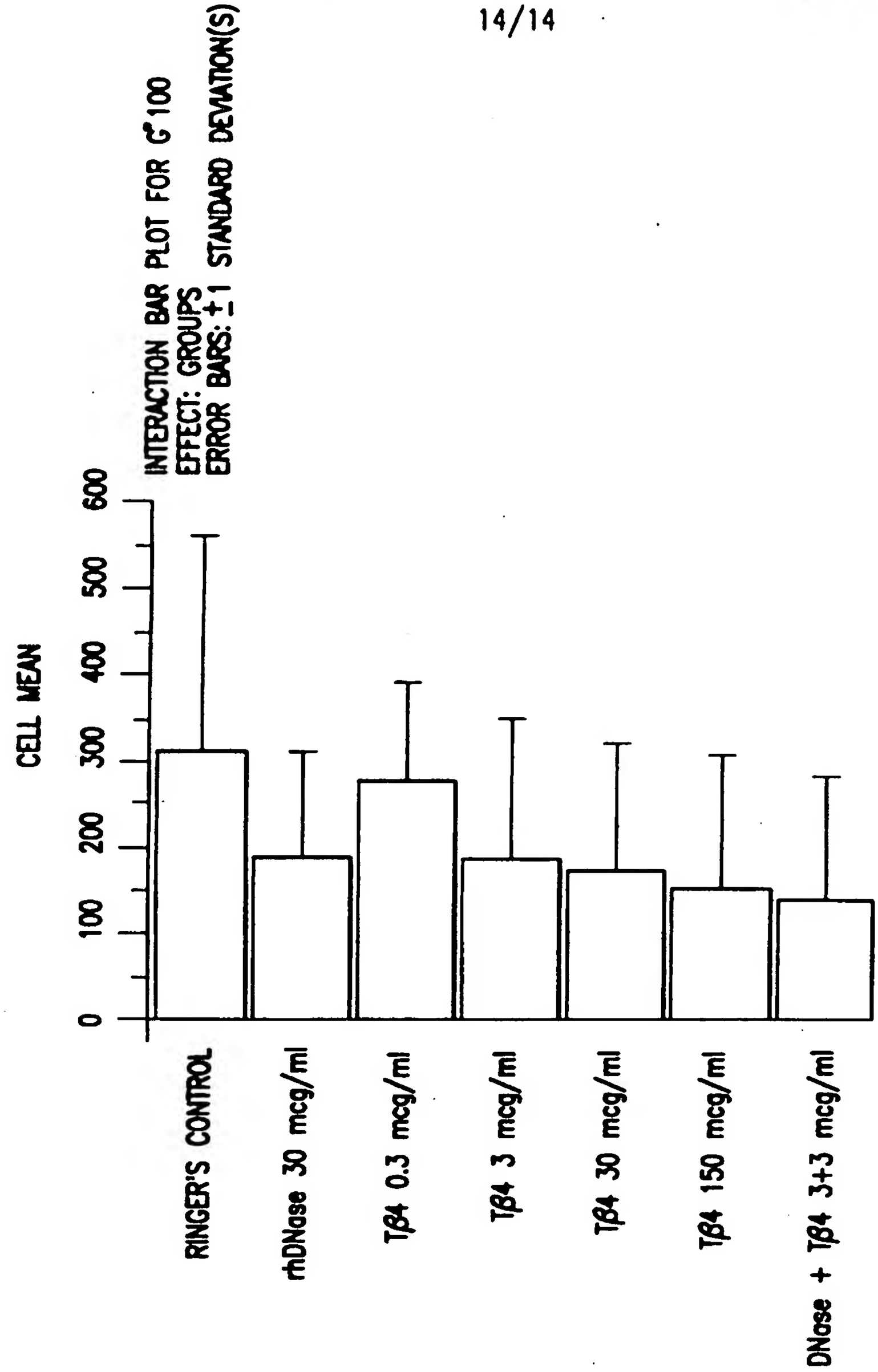
INTERACTION BAR PLOT FOR G'100
EFFECT: GROUPS
ERROR BARS: +1 STANDARD DEVIATION(S)
CELL MEAN
0
100
200
300
400
500
600
CELL
RINGER'S CONTROL
rhDNase 30 mcg/ml
Tβ4 0.3 mcg/ml
Tβ4 3 mcg/ml
Tβ4 30 mcg/ml
Tβ4 150 mcg/ml
DNase + Tβ4 3+3 mcg/ml

FIG.10

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/[redacted]

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :A61K 38/00, 38/32, 38/46
US CL :514/12, 4; 530/301, 324

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 4; 530/301, 324

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN (BIOSIS, CA, MEDLINE, WPIDS), APS
search terms: thymosin, sputum, DNAse I

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US, A, 4,297,296 (GOLDSTEIN ET AL) 27 October 1981, see entire document | 1-20 |
| P,Y | Pharmaceutical Research, Volume 11, No. 4, issued 1994, Cipolla et al, "Characterization of aerosols of human recombinant Deoxyribonuclease I (rhDNase) generated by jet nebulizers" pages 491-498, see, e.g., abstract. | 1-20 |
| Y | Science, Volume 263, Number 5149, issued 18 February 1994, Vasconcellos et al, "Reduction in viscosity of cystic fibrosis sputum in vitro by gelsolin", pages 969-971, see entire article. | 1-20 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 24 JANUARY 1996 | 06 FEB 1996 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>BENET PRICKRIL<br>Telephone No. (703) 308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Internatio[redacted]pplication No.
PCT/US[redacted]2543

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | New England Journal of Medicine, Volume 326, Number 26, Lee et al, "The extracellular actin-scavenger system and actin toxicity", pages 1335-1341, see entire article. | 1-20 |